Some Observations on Transpiration

Carlton C. Curtis

Variations in the rate of transpiration have been observed almost from the beginning of physiological research. These irregularities present a most puzzling problem since they appear in the results obtained by any method that may be employed to demonstrate the phenomena of transpiration and notwithstanding the fact that the external conditions may be apparently uniform and constant, I have been interested to take note of the records that have been made from year to year, when this subject comes up for experimentation in the laboratory with a view to determine whether there is any rhythm to be found in the fluctuations, and if so, whether this is related to the tension of fluids in the stem. In other words, to find out in what degree transpiration might be connected with the vital phenomena of the plant.

The table on the following page illustrates the character of the fluctuations and these examples are presented since they were obtained under as constant conditions as could be expected with natural light.

The amount of transpiration is expressed in milligrams obtained by hourly weighings beginning in the morning and running through the day, and below each series is given the changes of temperature and humidity while on the right hand of the table is noted the character of the day.

The intensity of the light may account in a certain degree for the maxima of the curves coming near the middle of the day although there was no apparent variation in the amount of illumination at that time of day. However, there are minor fluctuations in the curves quite independent of the slight variations of the climatic conditions and the light intensities and they are as likely to be at variance with any perceptible changes as in keeping with them. These irregularities may result in the rise or fall of the rate for a short period or again they may extend over longer periods. They are quite as appearent in the consecutive readings of potometers and especially in the device of Darwin's where a shoot

I. TRANSPIRATION CURVES IN DAYLIGHT

	6 A.M.	7	8	9	10	11	12	1 P.M.	2	3	4	5	6	
ABUTILON THOMPSONI														
	160	243	316	366	504	602	643	715	686	636	646	641	304	Cloudy with
Temperature	24				25			25.5		25		24		rain from 11
Hygrometer	24				25			21		23		23		to 5.
KALANCHOE GLAUCESCENS														
	12	22	28	32	36	48	64	57	39	34	36	26	18	Cloudy.
Temperature	26						27		28		27			
Hygrometer	30						28		26		28			
ANTHOLIZA AETHIOPICA														
	91	100	98	102	118	127	173	180	166	133	124	132	119	Snowing.
Temperature	20				21	22				21		19		
Hygrometer	33				30	30				34		36		
ACALYPHA HISPIDA														
	59	56	52	96	243	220	304	328	354	168	108	84	108	Raining,
KLEINIA FICOIDES														
	8	6	7	10	20	24	39	58	73	62	29	27	20	
Temperature	19		19			20		21				21		cl'ring 3.30
Hygrometer	55		54			52		50				48		but cloudy.
STACHYS GRANDIFLORA														
	26	30	40	74	67	84	120	115	99	99	60	36	39	Cloudy.
Temperature	17							17.5				17.5		
Hygrometer	42			44				43				46		
FICUS INFLECTA														
	81	91	136	82	63	195	244	229	239	150	62	57	50	Very dark
Temperature	19			20								21		in forenoon
Hygrometer	42			38								36		becoming foggy.

is sealed to a test-tube and the loss in weight is viewed with a horizontal microscope or in Gardner's experiment where the transpiration stream of a small shoot is viewed under a microscope. In both of these latter cases the transpiration rate can be examined in short intervals of time and we have a magnified view, so to speak, of the process. In all these methods the irregularity of the rate is the striking phenomenon. It is ever fluctuating and without apparent reason. It is exceptional to find consecutive readings that show a fixed ratio of increase or decrease. In the above table while the hourly weighings furnish singular irregularities they give an imperfect idea of the endless series of changes that are constantly going on.

In the curve found for *Abutilon*, the acceleration though continuing for eight hours (a very exceptional curve) increases very irregularly, *i. e.*, by 83 mg. 73, 50, 38, 98, 41, 72, and then declines by 29, 50, rises again by 11 and finally declines by 5,337—the last evidently a light effect. In the case of *Kleinia* and *Acalypha* an irregular decline follows for three hours when the rate for *Kleinia* accelerates for six hours and then declines, while in the case of *Acalypha* there is a rise for two hours followed by a decline for one hour, then a rise for three hours, then a decline for three hours, then a rise for one hour. The succeeding hour, not given in the table, showed a further decline, *i. e.*, 96 but still in excess of the rate at 5 o'clock. I was able to verify the character of these fluctuations during the summer of 1901 at the N. Y. Botanical Garden and it gives me pleasure to acknowledge the facilities for conducting the work that were placed at my disposal.

It would seem that these variations can only very indirectly be connected with external conditions and the facts would appear to warrant the conclusion that the phenomena here recorded are the expression of the vital processes at work in the plant. It should be said that in all the experiments recorded in this paper that care was taken to select thrifty potted plants and the jars were placed in large tin cans, the mouths being closed with tin covers and sealed with beeswax mixture after the manner suggested by Darwin. In this way a considerable volume of air was at the disposal of the roots and would serve to keep the plant in normal condition for some time. It was necessary to make the

weighings exactly at the end of the hour if the various measurements were to be of any value. It is apparent how imperfect a knowledge of the transpiration rate or how misleading may be the results when observations are taken at long or irregular intervals or for a short period and from such data drawing conclusions as to the rate.

For the purpose of comparison a number of experiments were conducted under more uniform conditions. The records found in Table II. were obtained in the dark room where the plants were exposed to an electric light of 900 candle power hung at a distance of 4 m. from the balance. The dark room was sufficiently light-tight to permit the handling of the most sensitive photographic plates without injury. When necessary the humidity was controlled in a degree by sprinkling the floor and the temperature rarely varied more than one degree. In this and the preceding table where the record of two plants are given at the same time two separate balances were used to avoid a possible error due to distributing the plants in moving them. Baranetsky's contention on this point has something of merit. Certainly with the most careful manipulation the operator runs a very considerable risk of disturbing the delicate balance in the plant and in a continued series of observations it is at least questionable whether the results obtained in this manner may always be relied upon. The plants in these experiments were usually placed in the dark room over night or for two or three hours before using. This permitted an adjustment to the conditions obtaining in the room and eliminated such after effects as have been noted by Schwendener and Sorauer. Buds and young leaves were cut off in many of the cases cited about one week before the experiment; in other instances such parts were sealed with a mixture of beeswax, tallow and linseed oil and then bound with sheet rubber. The interesting results obtained by Hoehnel on the relative transpiration rate of young and old leaves and similarly by Wiesner on the transpiration of developing stems and also of Sorauer on the relation of the formation of chlorophyll to the transpiration, renders it necessary in arriving at any conclusion as to what the plant is really doing to exclude such factors as may introduce variations that would furnish misleading results

or more probably quite nullify the value of the observations. As will be seen the measurements show an unmistakable curve characterized by minor fluctuations but demonstrating a grand curve with a maximum in the forenoon or near the middle of the day. In several instances I have noticed that transpiration was more active earlier in the day than was found in the first table. This may be due to the fact that the electric current was not turned on till 7 o'clock and the escape of the excess of intercellular vapor in the following hours overbalanced the normal transpiration under the feeble illumination. I regret that it was impossible to secure the curve for 24 hours under constant illumination. However, it will be seen that the curves for 12 hours bear a resemblance to the irregular daily curve of growth determined by Sachs. The absorption curves given by Vesque, also the curves of fluid tensions in the stem determined by Detmer and the periodicity of the exudation of fluids from cut stems, all show variations in keeping with the records set forth in Table II. It is interesting to note that these curves correspond in the main with those found in Table I. and show the same erratic character. Every plant appears to possess an individuality that becomes evident in these continued irregularities. In fact it has never been my experience to secure two measurements from the same plant that showed similar fluctuations, although special attention was given to placing them under the same conditions for two or more days between the tests.

While there may not necessarily be a constant relation between absorption, exudation and tension of fluids and the phenomena of growth, nevertheless, analogy and the general harmonizing of the ratios of these various rhythms with the curves of transpiration would seem to point unmistakably to the dependence of the latter process upon the vital activities of the plant. The marked irregularities in the rate are of special significance in this regard. As had been said they cannot be connected with any minor variations of the light, temperature or humidity—at least this statement would apply to many of the experiments where the instruments would show no appreciable fluctuations for hours at a time. Furthermore these results would naturally be expected where we consider Pfeffer's work on the energy of the cell. So many factors enter that the secretion and excretion are constantly

II. TRANSPIRATION CURVES UNDER ELECTRIC ILLUMINATION

	8 A.M.	9	10	11	12	1 P.M.	2	3	4	5	6	7
EUONYMUS JAPONICUS	38	67	105	167	116	82	110	108	92	89	72	
PELARGONIUM ZONALE	53	42	54	56	66	49	48	52	46	40	42	34
Temperature	25		26	27			26				25	
Hygrometer	28		30	28			31				30	
GARDENIA JASMINOIDES	27	36	57	60	65	60	63	55	33	38		
JUSTICIA ELEGANS	216	214	248	235	269	270	210	228	214	150		
Temperature	20		20.5				21		20.5			
Hygrometer	32		30				29		30			
FUCHSIA SPECIOSA	45	40	42	45	53	52	44	45	46	42		
AUCUBA JAPONICA	25	48	67	40	60	50	45	38	30	27		
Temperature.	22			21.5		22		22				
Hygrometer	37			36		36		38				
ACALYPHA HISPIDA	176	182	184	210	194	182	176	177	147	141	138	
Temperature	23				23					23.5		
Hygrometer	51				50					52		
FICUS ELASTICA	126	134	143	170	145	116	106	98	110	93		
Temperature	20		21				22					
Hygrometer	46		46		48		47					
SPARMANNIA	7	24	23	26	31	26	24	24	12	16		
Temperature	24.5				25				24.5			
Hygrometer	37				35				35			

subject to variation. Saturation, tension of gas, salts, food contents, metabolic processes, conditions of growth, all, acting independently of outward conditions, have in a degree a controlling influence on the cell activity and the expression of this complex interaction is found in the fluctuations. It is not intended to imply that transpiration is a purely physiological phenomena. The position of Nägeli is the only rational one—all physiological processes are dependent upon chemical and physical laws and every vital phenomenon goes on in accordance with these laws. This statement of Nägeli's made in 1861 would almost seem prophetic in the light of the knowledge on this subject of vital phenomena held to-day. We are accustomed to look upon the phenomena of transpiration as a purely physical process, largely, no doubt, because it is so intimately associated with the factors that govern evaporation. However, in this consideration we overlook the fundamental character of the physiological processes involved in transpiration and that are of the highest importance in growth, development and gaseous exchange. And it may be pointed out that the manner of action of light upon transpiration is by no means a settled problem, aside from the fact that it is intimately associated with the activities of the cell. Certainly the behavior of plants in light and darkness and the reaction of etiolated, chlorophylless and green plants to light would be difficult to explain on a purely physical basis. Again the after effects attending variations of illumination indicate interactions of a physiological character. If the vaporization of water in the plant is of a mechanical nature, having for its purpose the diffusion of the meager supply of salts required by the plants, then the process is not in keeping with the exceptional economy illustrated in the activities of the plant. It appears as rather remarkable when we consider the devices for the distribution of the slowly diffusing organized products, that the plant never hit upon an adaptation that would provide it with this relatively minute quantity of salts without putting it to the expenditure of so much energy. This supposition certainly ranks transpiration as perhaps the most wasteful operation in the vegetable kingdom.

The most variable factor in the external conditions of the experiments mentioned above was the variation in the percentage of

moisture. The temperature would remain constant for long periods but the humidity was subject to more considerable variations. I was interested to observe what results would follow changes of humidity. A rise of a few per cent. had no effect that could with certainty be recognized. The following figures will illustrate the result when the humidity was changed at 11 o'clock from 36 to 44.

III. EFFECT OF MOISTURE

	10 A. M.	11	12	1	2	Temp.	Hygrm.	Time.
Aucuba Japonica,	167	164	136	167	108	18.5	37	10
Richardia Africana,	142	138	135	112	106		36	11
Opuntia camnessa,	5	7	0	4	3		44	12
Begonia metalica,	98	117	93	80	70	19	44	1
Cycas revoluta,	70	70	37	29	20		44	2

The result of this very considerable change is unmistakable and is apparent in the increased irregularities of the curve. The variation in *Opuntia* is in keeping with what might be expected from a plant of so pronounced adaptive character. It should be stated, however, that the curves of several cacti, obtained under fairly uniform conditions, were very uneven. These results are such as to warrant the conclusion that no serious error was being introduced through the fluctuations of the hmidity.

A few measurements are given in Table IV., of the transpiration of plants in the dark. Occasionally there is apparent a rhythm corresponding to the daily periodicity, but on the other hand it not unfrequently happens that there may be an irregular decline from the start or the curve may be characterized by a rise or fall without reference to the period of the day. I have noticed on several occasions that pronounced maxima may be found early in the morning—between 5 and 9 o'clock.

Manifestly little importance is to be attached to the results obtained under such conditions inasmuch as they represent the work performed under practically pathological conditions and the results throw little light upon the real action of the plant. While the rate becomes more uniform as Kohl and Wiesner have pointed out there is also to be noted a marked decline and the plant is less responsive to the stimuli of light, all the phenomena, in fact, point to a cessation of the activities. The tissues become congested and it is apparent

IV. ABSENCE OF LIGHT

	6 A. M.	7	8	9	10	11	12	1 P. M.	2	3	4	5	6
FICUS INFLECTA,	58	71	64	52	53	96	70	110	68	54	56	48	
Temperature,	17.5		18						18.5				
Hygrometer,	51		50						49				
FICUS ELASTICA,		286	255	247	280	294	270	265	266	247	223	203	166
		17.5					17.5		18			18	
		40					41		40			39	
GARDENIA JASMINOIDES,		32	34	30	49	48	46	40	44	38	32	36	34
Temperature,		19.5			20		19.5	20.5				21	
Hygrometer,		37			37		38	37				36	
NERIUM OLEANDER,		53	60	57	59	83	75	64	62	56	45	40	
Temperature,		20	21				21				21		
Hygrometer,		46	45				44				44		
ACALYPHA HISPIDA,		90	88	84	68	76	85	93	119	86	78	80	84
Temperature,		18		19				20			20		
Hygrometer,		38		37				36			36		
JUSTICIA ELEGANS,	210	190	164	135	198	167	170	195	166	174	192	213	184
Temperature,	18			19					195				
Hygrometer,	36			36					33				

that this must lead to a disarrangement of the operation of the vital processes. I was particularly interested to note that the leaves in many cases decidedly changed their positions. They often appeared to flag as if from the loss of water. They were, however, perfectly rigid and the changed turgor tensions had caused the curvature of the petioles. In other instances the edges of the leaf turned strongly upwards. This was particularly the case in *Begonia* while in *Fuchsia* many of the leaves bent down, forming a half circle and in so rigid a leaf as that of *Ficus* a lateral twisting of the leaves was sometimes to be seen.

The amount of water transpired is rather surprising in view of the fact that we usually consider the stomata as closed in the dark. Very few authors have maintained that the contrary condition is the case. Considering the ratios that have been found by Garreau and others between the dorsal and ventral surfaces of leaves it appears impossible that the cuticular transpiration could equal the volume recorded in the table. Only one instance has been noticed where the plant seemed to have the power of closing the stomata. In the case of *Cycas revoluta* brought into the dark room at 7.45 a. m. weighings were made hourly from 12.45 on with the following results: loss of 7 mg., 25, then gained for four hours 3, 2, 3, 2, loss 2, 7, gain 2, 4, 2, 0, 2. Temp. 21 to 19, Hygrm. 32 to

36. Six days later the same plant, having been returned to the green house, gave from 10.10 a. m. these readings : gain 0, 6, 2, loss 4, 7, 4, 8. Temp. 22 to 23.5, Hygrm. 32 to 30. The changes in the temperature and humidity were not corollated with the fluctuations. The gain in weight may be due to a precipitation similar to that noted by Volken in certain plants.

I was interested to make some further investigations upon the relations between cuticular and intracellular transpiration. The results will be found in Table V. The stems and petioles of plants from which the immature leaves had been removed a week previous were carefully sealed with wax mixture and the transpiration rate determined in the dark for three hours. Then the under surface of the leaves were rubbed with the wax so as to thoroughly plug the stomata and then coated with a further coat of the wax. The figures indicate that the stomata were closed. This wax mixture, referred to above, seems to me to offer a much better sealing mixture that the commonly employed shellac preparations which may react upon the living cells.

V. CUTICULAR AND INTRACELLULAR TRANSPIRATION

	9 A. M.	10	11	12.30 P. M.	1.30	2.30	3.30	4.30	5.30
FICUS ELASTICA,	70	76	107	4	7	9	12	10	10
Temperature,	18.8			19.5			19		
Hygrometer,	50			52			48		
FICUS ELASTICA,		163	175	6	4	11	10	12	12
Temperature,		22						23	
Hygrometer,		48						45	
FICUS ELASTICA,	37	38	43	4	8	10	10	6	8
Temperature,	19.5			19			20		
Hygrometer,	47			46			43		

In the cases under consideration, where the diffusion owing to the nature of the leaf must be at a minimum, the results would appear to warrant the conclusion that the stomata are sufficiently open to permit diastomic transpiration during the night. The work of Blackman in demonstrating the slow diffusion of CO_2 through the cuticular membrane of leaves, likewise that of Morren in reference to the absorption of SO_2 are suggestive of a similar action for vapor gas. It would also be difficult to understand how respiration may in all cases be effected at night by diffusion. I have not been able to demonstrate that the stomata were open by

actual observation under the microscope but the rapid closing of the stoma in the case of plants standing in the shade suggests that possibly the balance between the tensions is so delicate that the least disturbance of the equilibrium, as in making the sections for examinations, may bring about the closing. It is also possible that, owing to the activity of the secreting cells and the accumulation of intracellular water, the stomata may open at varying periods and after the escape of the vapor close again. The rise in the rate after some one or more hours is perhaps due to the increase of water in the epidermal walls, induced by the turgor tensions. The work of Wiesner on the relative permeability of dry and moist membranes certainly points to such a conclusion. Comes has also demonstrated that the amount of water transpired from the two surfaces of the leaf when acting separately may be so increased that the total amount given off may exceed the normal transpiration of the leaf. The relatively large amount of water transpired in the third example after the sealing may be due to the quicker and stronger response in turgor tensions, thus bringing about a more rapid saturation of the epidermal membrane and there may also be an interaction on the stomatal apparatus.

Another feature of transpiration that emphasizes the intimate relation existing between the process and the vital action of the plant is the periodicity to be seen in the opening and closing of the stomata. Darwin has recently shown that the stomata have acquired the habit of opening more readily in the morning under the influence of light than in the afternoon. This is very strikingly shown in the experiments that are recorded in table VI.

The plants were given one hour illumination at various hours in the forenoon, indicated by the larger type, and for the same length of time in the afternoon. In the two last measurements the electric light was used. I have not a sufficient number of experiments at hand to warrant a conclusion as to the hour when the greatest response may be obtained and, owing to the very uncertain quality of the light from day to day as well as the influence of after effects, this would be a difficult if not impossible task. The results show in a very striking manner that there is a pronounced periodicity in the stomata of the plants under consideration. The stomata appear to open quicker and wider in the

VI. PERIODICITY OF THE STOMATA

IV.

	9 A.M.	10	11	12	1 P.M.	2	3	4	5	6	7
GERANIUM ZONALE,	56	60	*107*	78	67	49	44	*64*	46	40	
GERANIUM ZONALE,	60	68	*128*	78	80	62	45	*96*	55	36	
Temperature,		23					23.5		23		
Hygrometer,		47				47			49		
GERANIUM ZONALE,	19	23	24	*68*	46	28	24	*49*	28	25	23
Temperature,		25				24.5			24		
Hygrometer,		36			32				32		
GARDENIA FLORIDA,		48	*75*	65	62	32	40	*65*	40	27	30
JUSTICIA ELEGANS,		306	568	460	322	302	270	*302*	275	255	217
Temperature,		22.5						22		21.5	
Hygrometer,		48						51		53	
FUCHSIA SPECIOSA,	50	*103*	84	48	58	58	53	*71*	50	48	
Temperature,		20					21		20		
Hygrometer,		56					54		55		
AUCUBA JAPONICA,	43	43	*48*	44	46	43	42	*44*	40	40	
Temperature,		19.5						19.5			
Hygrometer,		59						58			
JUSTICIA ELEGANS,			196	175	180	*210*	176	167	150	*154*	136
Temperature,			20.5		20			19.5			
Hygrometer,			43		43			44			

morning. At least this might be inferred from the volume transpired and the reverse is true in the afternoon. Of interest also is the relation of the after effect. The volume for one or more hours following the period of illumination in the morning is in excess of the volume preceding the period of illumination. In the afternoon this relation may not hold good or there may be a slight excess in the volume following illumination over that of the preceding period. While the periodicity would assist in bringing about this relation it is evident that under the conditions of experimentation it could not produce so constant a rhythm.

One feature of this work that has been constantly before me is the extreme sensitiveness of the plant and the consequent necessity for the greatest care in avoiding the introduction of disturbing influences. Burgerstein's suggestion that transpiration phenomena, when possible, should be studied by weighing would appear to promise the most trustworthy results. When we consider the complex relations existing in the plant and their varied reaction to external conditions it would appear that much of the work that had been done upon this subject had been prosecuted

under exceptionally brutal conditions. Devices which employ shoots or portions of the plant are of great value for demonstrations rather than as a means for the exact determination of actual processes. This remark may also apply to the measurements obtained by condensation or absorption of vapor. Work conducted under so artificial and unusual conditions can only give uncertain results.

SUMMARY

On cloudy and stormy days when the intensity of the light is quite uniform or at least not in keeping with the time of day, the transpiration curve shows a pronounced maximum near midday and it is also characterized by minor fluctuations that occur independently of climatic changes.

An illumination of an electric light of 900 candle-power under uniform external conditions demonstrated a periodicity in the transpiration of several plants that corresponds in the main with the curves determined on cloudy days. The harmonizing of the rhythms of absorption, exudation and tension of fluids and the rate of growth is suggestive of the vital character of transpiration.

Variations of a few per cent. in the humidity of the atmosphere produced no change in the amount of transpiration that could be determined with certainty. An increase of 8 per cent. resulted in a marked drop in the rate.

The curve in the dark sometimes was in keeping with the rhythm found under constant illumination but more frequently it is characterized by variations quite out of keeping with the periodicity of light. There is evidently a cessation of the vital action of the plant and the retarding of the rate together with its more pronounced regularity is in keeping with the loss of tone manifest in the plant.

The very considerable volume transpired in the dark indicates that the stomata may be sufficiently open to allow the escape of vapor. This may be brought about in connection with the interchange of the gases in respiration or possibly by the independent action of certain stomata that are especially affected by the increased turgor tensions or vapor tensions of intercellular transpiration. Only in the case of *Cycas revoluta* did the amount of transpiration appear in keeping with the ratios determined for cuticular transpiration.

This supposition became more manifest when the stems and petioles of several plants of *Ficus elastica* were sealed and the rate of transpiration in the dark was measured for three hours after which the stomatal surfaces were sealed and the rate of cuticular transpiration was determined for four hours. This amounted to from 3.6 to 10 per cent. of the former volume.

The physiological character of transpiration is also indicated by the periodicity of the opening and closing of the stomata. They are more responsive to the stimulus of light in the morning than in the afternoon and the more considerable physiological activity in the morning is manifest in the more pronounced after-effect following the illumination in the forenoon.